

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



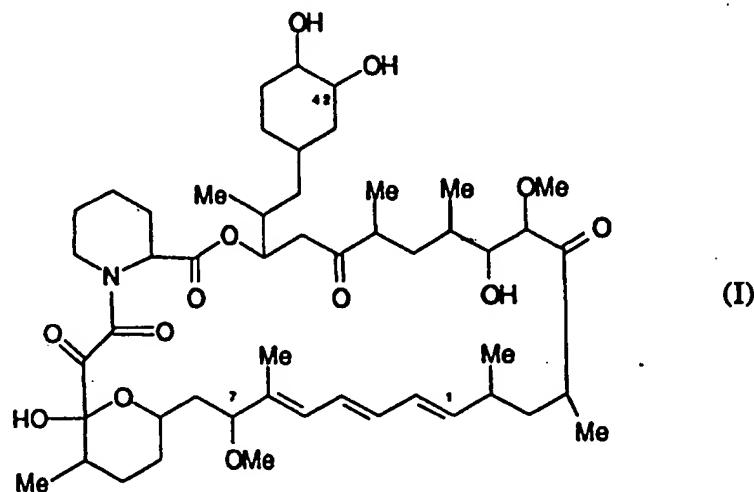
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 498/18, A61K 31/435, C12P 17/18, C12R 1/465		A1	(11) International Publication Number: WO 95/04060 (43) International Publication Date: 9 February 1995 (09.02.95)
(21) International Application Number: PCT/GB94/01600			(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(22) International Filing Date: 25 July 1994 (25.07.94)			
(30) Priority Data: 9315914.3 31 July 1993 (31.07.93)		GB	Published <i>With international search report.</i>
(71) Applicant (for all designated States except US): SMITHKLINE BEECHAM PLC [GB/GB]; New Horizons Court, Brentford, Middlesex TW8 9EP (GB).			
(72) Inventor; and			
(75) Inventor/Applicant (for US only): FORD, Brian, Douglas [GB/GB]; SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ (GB).			
(74) Agent: RUSSELL, Brian, John; SmithKline Beecham plc, Corporate Intellectual Property, SB House, Great West Road, Brentford, Middlesex TW8 9BD (GB).			

(54) Title: RAPAMYCIN DERIVATIVE WITH ANTIMICROBIAL, ANTICANCER AND IMMUNOMODULATION ACTIVITY

(57) Abstract

A compound of formula (I) is obtainable by the cultivation of a microorganism from the genus *Streptomyces*, such as *Streptomyces* NCIMB 40515, in the presence of rapamycin, and recovery thereof from the culture medium. The compound, and pharmaceutical compositions containing it, is useful as an immunomodulatory agent, as an anticancer agent, or in the treatment of microbial infections in animals including humans.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Larvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

RAPAMYCIN DERIVATIVE WITH ANTIMICROBIAL, ANTICANCER AND IMMUNOMODULATION ACTIVITY

The present invention relates to a novel compound and derivatives thereof, to processes for their production, to pharmaceutical formulations 5 containing them, to their use in medical therapy, particularly in the treatment of microbial infections, and also to their use as immuno modulatory agents.

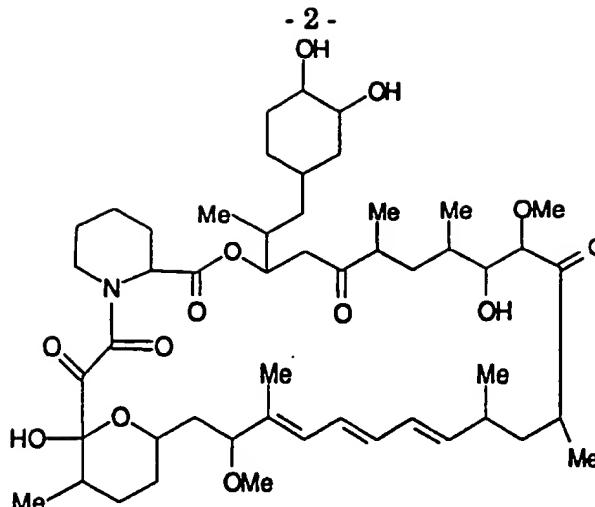
Rapamycin is a known compound and was first isolated as an extract of 10 the fungus Streptomyces hygroscopicus and reported to have antifungal activity (British Patent 1436447). Subsequently rapamycin has been implicated as an immunosuppressant (Martel R.R. *et al* Can. J. Physiol. Pharmacol. 55, 48-51, 1977).

15 A large number of microorganisms have been found to produce a variety of compounds which have subsequently been isolated and have been shown to possess useful therapeutic properties. Novel compounds have also been obtained by the incubation or cultivation of a microorganism in the presence of known compounds. One such new compound is
20 42-O-demethylrapamycin. This novel compound has been found to have useful antimicrobial and anticancer and immunomodulation activity.

Accordingly the present invention provides 42-O-demethylrapamycin and derivatives thereof.

25 The invention in a second aspect, further provides a process for the production of 42-O-demethylrapamycin which comprises contacting a microorganism with rapamycin and subsequently isolating 42-O-demethylrapamycin or derivatives thereof from the incubation.

30 42-O-demethylrapamycin is believed to have the structure shown in formula (I):



(I)

This compound is referred to herein as 42-O-demethylrapamycin

5 according to the numbering system of J. Findlay *et al.*, Can. J. Chem. (1980) **58**, 579. However according to the more recent numbering system of J. Mc Alpine *et al.*, J. Antibiotics (1991) **44**, 688 this would be known as 39-O-demethylrapamycin.

10 Following the numbering system according to Chemical Abstracts (11th Cumulative Index 1982-86 page 60719CS), the compound of the present invention would be called 41-O-demethylrapamycin.

The compound in formula (I) has the following characteristics:

15 i) it has an apparent molecular weight of 899 by fast atom bombardment (FAB) mass spectroscopy;

20 ii) it is obtainable by the cultivation of a microorganism from the genus *Streptomyces*, in the presence of rapamycin and the recovery of 42-O-demethylrapamycin or a derivative thereof from the culture medium;

25 iii) $^{13}\text{CNMR}$ spectroscopy reveals 50 carbons in the molecule;

iv) it shows antifungal activity;

v) it shows immunomodulatory activity.

- 3 -

The term 'cultivation' (and derivatives of that term) as used herein means the deliberate aerobic growth of an organism in the presence of assimilable sources of carbon, nitrogen, sulphur and mineral salts. Such aerobic growth may take place in a solid or semi-solid nutritive medium, 5 or in a liquid medium in which the nutrients are dissolved or suspended. The cultivation may take place on an aerobic surface or by submerged culture. The nutritive medium may be composed of complex nutrients or may be chemically defined.

10 It has been found that suitable microorganisms for use in the process according to the invention include bacterial strains belonging to the genus Streptomyces which are capable of elaborating 42-O-demethylrapamycin. It has further been found that an example of such a strain is sp. NCIMB 40515, which has been isolated from nature and also mutants thereof.

15 The term 'mutant' as used herein includes any mutant strain which arises spontaneously or through the effect of an external agent whether that agent is applied deliberately or otherwise. Suitable methods of producing mutant strains including those outlined by H.I. Adler in 'Techniques for 20 the Development of Microorganisms' in 'Radiation and Radioisotopes for Industrial Microorganisms', Proceedings of a Symposium, Vienna, 1973, page 241, International Atomic Energy Authority, and these include:

(i) Ionizing radiation (e.g. X-rays and γ -rays), u.v. light, u.v. light plus 25 a photosensitizing agents (e.g. 8-methoxysoralen), nitrous acid, hydroxylamine, pyrimidine base analogues (e.g. 5-bromouracil), acridines, alkylating agents (e.g. mustard gas, ethyl-methane sulphonate), hydrogen peroxide, phenols, formaldehyde, heat, and

30 (ii) Genetic techniques, including, for example, recombination, transformation, transduction, lysogenisation, lysogenic conversion, protoplast fusion and selective techniques for spontaneous mutants.

Using the methods of Becker B. Lechevalier M.P., Gordon R.E., 35 Lechevalier H.A., 1964, Appl. Microbiol. 12, 421-423 and Williams S.T., Goodfellow M, Wellington E.M.H., Vickers J.C., Alderson. G., Sneath P.H.A., Sackin M.J., and Mortimer M. 1983 J. Gen. Microbiol. 129, 1815-1830, Sp. NCIMB 40515 has been identified as a previously unreported,

- 4 -

atypical, strain of *Streptomyces* and therefore also forms a part of the present invention, particularly in biologically pure form. It has been deposited at the National Collections of Industrial and Marine Bacteria Ltd. (N.C.I.M.B), Aberdeen, Scotland under number NCIMB 40515 on 7th 5 July 1992.

The medium for cultivating sp. NCIMB 40515 suitably contains sources of assimilable carbon and assimilable nitrogen together with inorganic salts. Suitable sources of nitrogen include yeast extract, soyabean flour, meat 10 extract, cottonseed, flour, malt, distillers dried solubles, amino acids, protein hydrolysates and ammonium and nitrate nitrogen. Suitable carbon sources include glucose, lactose, maltose, starch and glycerol. Suitably the culture medium also includes alkali metal ions (for example, sodium), halogen ions (for example, chloride), and alkaline earth metal 15 ions (for example calcium and magnesium), as well as trace elements such as iron and cobalt.

The cultivation may suitably be effected at a temperature of about 20 to 35°C, advantageously 20 to 30°C, and the culture may suitably be 20 contacted with rapamycin for up to 7 days, advantageously about 3 to 5 days, in order to give an optimum yield of the desired product after isolation for example as described below.

The desired product or a derivative thereof may be isolated from the 25 culture medium and worked up and purified using conventional techniques for such compounds. All such isolation and purification procedures may conveniently be effected at cool to ambient temperature, for example at a temperature within the range of from 4 to 40°C, conveniently from 20 to 35°C.

30 The desired compound may readily be identified in a routine manner by testing for biological activity and/or by monitoring the h.p.l.c. retention time.

35 Suitably, the separation procedure may include a high-performance liquid chromatography step, preferably as the last step. Elution may be effected using aqueous methanol.

- 5 -

42-O-demethylrapamycin and its derivatives may be crystalline or non-crystalline and, if crystalline, may optionally be hydrated or solvated.

The derivatives are preferably pharmaceutically acceptable derivatives.

5 Derivatives may include salts, with pharmaceutically acceptable counter ions.

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 10 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight. An impure or less pure form of a compound according to the invention may, for example, be used in the preparation of a more pure form of the same compound or of a 15 related compound (for example a corresponding derivative) suitable for pharmaceutical use.

42-O-demethylrapamycin and its pharmaceutically acceptable derivatives have antifungal activity and are useful for the prophylactic and 20 therapeutic treatment of fungal infections in animals, especially mammals, including humans, in particular humans and domesticated animals (including farm animals). The compounds may be used for the treatment of topical fungal infections in man caused by, among other organisms, species of Candida (eg Candida Albicans), Trichophyton (eg 25 Trichophyton mentagrophytes), Microsporum (eg Microsporum gypseum) or Epidermophyton or in mucosal infections caused by Candida Albicans (e.g. thrush and vaginal candidiasis). They may also be used in the treatment of systemic fungal infections caused by, for example Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus, Coccidioides, 30 Paracoccidioides, Histoplasma or Blastomyces spp. They may also be of use in treating eumycotic mycetoma, chromoblastomycosis and phycomycosis.

42-O-demethylrapamycin and its pharmaceutically acceptable derivatives are also active as an immunomodulatory agents. The term 35 "immunomodulatory agent" as used herein means that the compound of the invention is capable of inducing immune suppression by inhibiting T (and B) cell responses in vitro and/or by producing a statistically significant decrease in the inflammation system response medicated

- 6 -

secondary lesion in the adjuvant induced arthritis. Indications for therapy include, but are not limited to, the treatment of the following disease states: rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis, acute transplantation/grant rejection, myasthenia gravis, progressive systemic sclerosis, multiple myeloma, atopic dermatitis, hyperimmunoglobulin E, hepatitis B antigen negative chronic active hepatitis, Hashimoto's thyroiditis, Familial Mediterranean fever, Grave's disease, autoimmune hemolytic anemia, primary biliary cirrhosis, inflammatory bowel disease, insulin dependent diabetes mellitus.

10 42-O-demethylrapamycin and its pharmaceutically acceptable derivatives should also have activity against carcinogenic tumours. More specifically, the compounds should be useful for reducing tumour size, inhibiting tumour growth and/or prolonging the survival time of tumour-bearing animals.

15 Accordingly the invention provides 42-O-demethylrapamycin or derivatives thereof for use in medical therapy, in particular for use as an antifungal agent or immunomodulatory agent, or as an agent against carcinogenic tumours.

20 The invention further provides a method of treating a human or animal suffering from a fungal infection by the administration of an effective amount of 42-O-demethylrapamycin or derivative thereof.

25 Moreover, the invention provides a method of treating a human or animal in need of immunomodulation by administration of an effective amount of 42-O-demethylrapamycin or derivative thereof.

30 The invention also provides a method of treating carcinogenic tumours in a human or animal comprising administering to such human or animal an effective, non-toxic amount of 42-O-demethylrapamycin or derivative thereof.

35 The invention further provides a pharmaceutical composition comprising a compound of the formula (I) or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable diluent or carrier. The composition is preferably for human use in tablet, capsule, injectable

or cream form.

For human use 42-O-demethylrapamycin or derivatives thereof can be administered alone, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of a tablet containing such excipients as starch or lactose, or in a capsule or ovule either alone or in admixture with excipients, or in the form of an elixir or suspension containing a flavouring or colouring agent. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile solutions which may contain other substances, for example, enough salts or glucose to make the solution isotonic.

For oral and parenteral administration to human patients suffering from a fungal infection, it is expected that the daily dosage level of the antifungal compounds of formula (I) will be from 0.05 to 100 preferably 0.1 to 10 mg/kg (in divided doses) when administered by either the oral or parenteral route. Thus tablets or capsules of the compounds can be expected to contain from 5 mg to 0.5 g of active compound for administration singly or two or more at a time as appropriate. The physician in any event will determine the actual dosage which will be most suitable for an individual patient and will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

Equally for a human patient in need of immunomodulation the daily parenteral or oral dosage regimen for the compound or derivative thereof will preferably be from 0.1 mg/kg to 30 mg/kg.

One skilled in the art would be able, by routine experimentation, to determine what an effective, non-toxic amount of compound or derivative thereof would be for the purpose of treating carcinogenic tumours. Generally, however, an effective dosage is expected to be in the range of about 0.05 to 100 milligrams per kilogram body weight per day.

No unacceptable toxicological effects are expected when the compound is administered in the above mentioned dosage ranges.

- 5 The compounds and compositions according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antifungal, anticancer or immunomodulatory agent.
- 10 The compounds and tablets and capsules for oral administration may be in unit dosage form, and may contain conventional excipients including, for example, binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, 15 for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; and pharmaceutically acceptable wetting agents, for example sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice.
- 20 Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain 25 conventional additives, including, for example, suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles (which may include 30 edible oils), for example almond oil, oily esters (for example glycerine), propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and, if desired, conventional flavouring and colour agents.
- 35 Compositions according to the invention intended for topical administration may, for example, be in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, impregnated dressings, and aerosols, and may contain appropriate conventional additives, including,

- 9 -

for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may

5 constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

Compositions according to the invention may be formulated as suppositories, which may contain conventional suppository bases, for

10 example cocoa-butter or other glycerides.

Compositions according to the invention intended for parenteral administration may conveniently be in fluid unit dosage forms, which may be prepared utilizing the compound and a sterile vehicle, propylene glycol.

15 The compound, depending on the vehicle and concentration used, may be either suspended or dissolved in the vehicle. Parenteral suspensions may be prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may instead be

20 sterilised by exposure to ethylene oxide before being suspended in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in such suspensions in order to facilitate uniform distribution of the compound.

25 Compositions according to the invention may also be administered by inhalation. By "inhalation" is meant intranasal and oral inhalation administration. Appropriate dosage forms for such administration such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques.

30 The following examples serve to illustrate the present invention.

EXAMPLE 1

35 **Preparation of 42-O-demethylrapamycin**

A culture capable of producing 42-O-demethylrapamycin from rapamycin has been classified as Streptomyces sp. and has been deposited in the

- 10 -

National Collection of Industrial and Marine Bacteria, 23, St. Machar Drive, Aberdeen AB2 1RY, Scotland, UK. under the accession number NCIMB 40515.

5 Cultivation

Each of five 500ml flasks containing 100ml M2 medium [arkasoy, 10g/l; glycerol, 20g/l; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.005g/l; $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1g/l; FeCl_3 , 0.03g/l; ZnCl_2 , 0.005g/l; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.005g/l; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.005g/l pH 6.6 10 unadjusted] were inoculated with 2 plugs of agar from well growing cultures on A3 agar [yeast extract, 5g/l; malt extract, 10g/l; glycerol, 10g/l; peptone soya, 5g/l; agar No.3, 20g/l; pH 6.5] in petri dishes. Alternatively a large loopful of well growing culture from a petri dish was mixed with 3ml of Tween 80 and the total contents added to 100ml M2 media in a 500 15 ml flask, grown at 28°C, 240 rpm for 3 days, 1ml of the culture broth is then used to inoculate 100ml M2 media in each of the five 500ml flasks. The flasks were then grown at 28°C and 240 rpm.

Incubation

20 After 5 days, rapamycin (20mg) as a solution in acetone (3.5ml) was added to each flask. Rapamycin can be obtained from a rapamycin-producing culture NCIMB 40319 deposited on 14 September 1990, or by culturing a rapamycin producing organism eg NRRL 5491 as disclosed in US Patent 25 3,929,992 issued 30 December 1975, the entire disclosure of which is hereby incorporated by reference. Flasks were incubated at 28°C, 240rpm for about 20h.

Isolation procedure

30 Solvent extraction

The contents of the flasks was bulked and adjusted to pH4 with dilute sulphuric acid. 500ml of dichloromethane was added and the mixture 35 stirred for 2h, the organic solvent phase was recovered separating the phases by centrifugation and a further 200ml of dichloromethane added and stirred for 1h. The organic solvent phases were combined and concentrated in vacuo to an oil. To the oil was added 100ml methanol, the

- 11 -

methanol extract filtered and the filtrate concentrated to an oil in vacuo.

Silica chromatography

5 The oil was loaded on a Kieselgel 60 (70-230 mesh) column (25 x 50mm) packed in acetone:hexane (15:85). After loading, the column was eluted with a step gradient of acetone-hexane. Fractions eluted after 35:65 acetone:hexane contain 42-O-demethylrapamycin. These were combined and reduced to dryness in vacuo and stored at -20°C.

10

Preparative hplc

15 The stored solid was dissolved in 500µl methanol and 100µl portions injected separately onto a reverse phase Microsorb C-18 column and pre column (21.4 mm x 25cm and 21.4 x 5cm) (Rainin Instruments USA). After injection elution continued with 78:22 methanol:H₂O, at 6ml/minute and was monitored for UV absorbance at 278nm. Fractions containing the object compound from a total of 5 injections were pooled, concentrated in vacuo to remove the methanol and freeze dried.

20 Fractions containing the object compound were analysed by reverse phase hplc using a Spherisorb S10ODS2 (PhaseSep) column (25cm x 4.6mm) and a Waters pre-column. The column was monitored by UV absorption at 278nm and eluted with 78:22 methanol-water at 2ml/minute. Under these conditions the object compound had a retention time of 6.8 minutes

25 (differing from rapamycin, retention time of 8.4 minutes).

Spectroscopic Data

30 The resulting compound was characterised by mass spectroscopy (FAB)[M + Na]⁺ = 922 and by proton and ¹³C nuclear magnetic resonance spectroscopy (see Table 1 below). UV spectroscopy shows UV λ_{max} in acetone at 275 nm.

- 12 -
Table 1

Spectroscopic Data for 42-O-Demethylrapamycin:

5 MS (FAB/NaCl) m/z 922 (M+Na)⁺

1H-NMR (CDCl₃, 400 MHz, 4:1 mixture of trans: cis amide rotamers; data for the trans-rotamer):

ppm	assign	multipl	J's (Hz)
6.383	H-4	dd	14.7, 10.5
6.310	H-3	dd	14.7, 9.8
6.134	H-2	dd	15.0, 9.8
5.970	H-5	d	10.5
5.518	H-1	dd	15.0, 9.0
5.407	H-26	d	10.3
5.273	H-20	d	5.0
5.152	H-22	q	5.8
4.618	OH-13	s	-
4.197	H-28	br d	5.0
3.89	H-9	m	-
3.782	H-29	d	5.3
3.647	H-7	t	7.3
3.515	H-16	br d	11.9
3.338	MeO-29	s	-

10 3.4-3.25 overlapping multiplet of H-25, H-42 and H-43

3.134	MeO-7	s	-
2.710	H-23	dd	16.8, 5.8
2.587	H-23	dd	16.8, 5.7
1.758	Me-27	s	-
1.655	Me-6	s	-
1.085	Me-25	d	6.7
1.051	Me-33	d	6.4
0.985	Me-31	d	6.5
0.942	Me-12	d	6.6
0.905	Me-38	d	6.7

EXAMPLE 2**Bioactivity of 42-O-demethylrapamycin**

5 The compound was analysed for antifungal and immunosuppressive activity using the following bioassays:

A. Assay for antifungal activity

10 Yeast organism (*Saccharomyces cerevisiae*) in logarithmic growth were plated on complete agar medium (YPD). Compounds dissolved in an appropriate aqueous or organic solvent were placed in wells punched in the agar. Plates were incubated for 48 hours and zones of inhibition were measured. The potency of compounds were quantified by regression analysis of plots of inhibition zone versus the log of drug concentration.

15

B. Mitogenesis Assay for Immunosuppressive Activity.

20 Spleen cells for BDF1 female mice were established in RPMI with 10% fetal calf serum at 5×10^6 /mL. One hundred mL aliquots of this suspension (5×10^5 cells) were dispensed into 96-well round-bottomed microtiter plates (Linbro, Flow Laboratories). Concanavalin A (5 μ g/ml) was added as the mitogenic stimulus, and the final volume in the microtiter wells was adjusted to 200 μ L with RPMI. Cell cultures were

25 incubated for 72 hours at 37°C in a 5% CO₂ atmosphere and pulsed with 0,5 μ Ci ³H-thymidine (specific activity 2.00 Ci/mole) for the last 18 hours of the 72 hours culture. The cells were harvested on an automated multiple sample harvester and cell-associated radioactivity counted in a Beckman liquid scintillation counter. The results were expressed as the

30 mean values derived from quadruplicate measurements. Cell viability was determined by trypan blue exclusion after 72 hours of incubation. Compounds to be tested were added to the microtiter plates at the appropriate dilutions prior to the addition of cells.

35 Results of these two assays for compounds of this invention are provided in Table 2.

Tabl 2

Compound	a Antifungal Activity	b Mitogenesis
Control (rapamycin)	0.0061	1 ± 1
42-O-demethylrapamycin	0.012 0.013	3 -

5 a Assay : (IC₁₂, µg/ml)
b Assay : (IC₅₀, nM)

EXAMPLE 3

10 COMPOSITION EXAMPLES A - H

A - CAPSULE COMPOSITION

15 A pharmaceutical composition of this invention in the form of a capsule is prepared by filling a standard two-piece hard gelatin capsule with 50 mg of a compound of the invention, in powdered form, 100 mg of lactose, 32 mg of talc and 8mg of magnesium stearate.

B - INJECTABLE PARENTERAL COMPOSITION

20 A pharmaceutical composition of this invention in a form suitable for administration by injection is prepared by stirring 1.5% by weight of a compound of the invention in 10% by volume propylene glycol and water. The solution is sterilized by filtration.

25 C - OINTMENT COMPOSITION

Compound of the invention 1.0g
White soft paraffin to 100.0g
30 The compound of the invention is dispersed in a small volume of the vehicle and granually incorporated into the bulk of the vehicle of produce

- 15 -

a smooth, homogeneous product. Collapsible metal tubes are then filled with the dispersion.

D - TOPICAL CREAM COMPOSITION

5

Compound of the invention 1.0g
Polawax GP 200 20.0g
Lanolin Anhydrous 2.0g
White Beeswax 2.5g

10 Methyl hydroxybenzoate 0.1g

Distilled Water to 100.0g

The polawax, beeswax and lanolin are heated together at 60°C. The compound of the invention is then added and dispersed throughout, and the composition is allowed to cool with slow speed stirring.

15

E - TOPICAL LOTION COMPOSITION

Compound of the invention 1.0g

Sorbitan Monolaurate 0.6g

20 Polysorbate 20 0.6g

Cetostearyl Alcohol 1.2g

Glycerin 6.0g

Methyl Hydroxybenzoate 0.2g

Purified Water B.P. to 100.00ml

25 The methyl hydroxybenzoate and glycerin are dissolved in 70ml of the water at 75. The sorbitan monolaurate, polysorbate 20 and cetostearyl alcohol are melted together at 75 C and added to the aqueous solution.

The resulting emulsion is homogenized, allowed to cool with continuous stirring and the compound of the invention is added as a suspension in

30 the remaining water. The whole suspension is stirred until homogenized.

F - EYE DROP COMPOSITION

Compound of the invention 0.5g

Methyl Hydroxybenzoate 0.01g

35 Propyl Hydrobenzoate 0.04g

Purified water B.P. to 100.00 ml (B.P.= British Pharmacopia)

The methyl and propyl hydroxybenzoates are dissolved in 70ml purified water at 75°C and the resulting solution is allowed to cool. The compound

- 16 -

of the invention is then added, and the solution is sterilized by filtration through a membrane filter (0.22 μ m pore size) and packed aseptically into suitable sterile containers.

5 G - COMPOSITION FOR ADMINISTRATION BY INHALATION

For an aerosol container by a capacity of 15-20ml: Mix 10mg of a compound of the invention with 0.2-0.2% of a lubricating agent, such as polysorbate 85 or oleic acid, and disperse such mixture in a propellant, 10 such as freon, preferably in a combination of (1,2 dichlorotetrafluoroethane) and difluorochloromethane and put into an appropriate aerosol container adapter for either transnasal or oral inhalation administration.

H - COMPOSITION FOR ADMINISTRATION BY INHALATION

15 For an aerosol container with a capacity of 15-20ml: Dissolve 10mg of a compound of the invention in ethanol (6-8ml), add 0.1-0.2% of a lubricating agent, such as polysorbate 85 or oleic acid; and disperse such in a propellant, such as freon, preferably a combination of (1,2 20 dichlorotetrafluoroethane) and difluorochloromethane, and put into an appropriate aerosol container adapter for either intranasal or oral inhalation administration.

25

30

35

International Application No: PCT/

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page 4, line 4 of the description 1**A. IDENTIFICATION OF DEPOSIT¹**Further deposits are identified on an additional sheet Name of depository institution ¹NATIONAL COLLECTION OF INDUSTRIAL AND MARINE BACTERIA
LIMITEDAddress of depository institution (including postal code and country) ¹Torry Research Station, P.O. Box 31, 135 Abbey Road
Aberdeen AB9 8DG, United KingdomDate of deposit ¹

7 July 1992

Accession Number ¹

NCIMB 40515

B. ADDITIONAL INDICATIONS¹ (leave blank if not applicable). This information is continued on a separate attached sheet

In respect of those designations in which a European Patent is sought, a sample of the deposited micro-organism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE¹ (If the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS¹** (leave blank if not applicable)The indications listed below will be submitted to the International Bureau later² (Specify the general nature of the indications e.g., "Accession Number of Deposit")**E.** This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

Paul Ranson The date of receipt (from the applicant) by the International Bureau ¹

W86

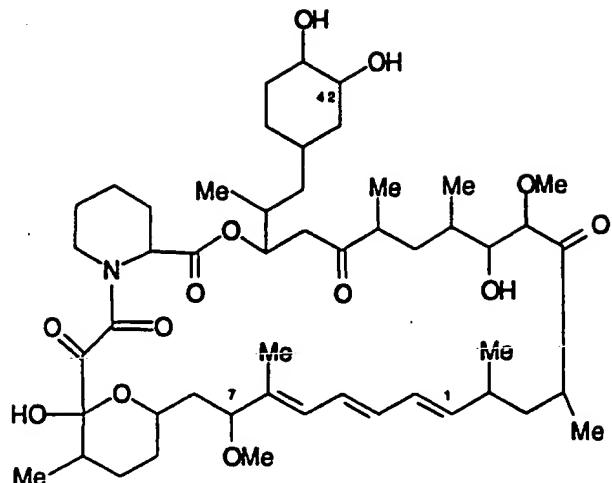
(Authorized Officer)

- 18 -

Claims

1. A compound of formula (I):

5



(I)

which is 42-O-demethylrapamycin, or a derivative thereof.

10

2. A compound characterised by having the following characteristics:

- 15 i) it has an apparent molecular weight of 899 by fast atom bombardment (FAB) mass spectroscopy;
- ii) it has 50 carbons in the molecule according to ^{13}C NMR spectroscopy;
- iii) it shows antifungal activity;
- iv) it shows immunomodulatory activity;

20

said compound being obtainable by the cultivation of a microorganism from the genus *Streptomyces* in the presence of rapamycin, and recovery thereof from the culture medium.

25

3. A process for the production of a compound according to claim 1, which comprises contacting a microorganism with rapamycin and subsequently isolating 42-O-demethylrapamycin, or a derivative thereof, from the incubation.

- 19 -

4. A process according to claim 3, which comprises separating the substance or compound or a derivative thereof from a solution thereof in admixture with other antibacterially active substances and/or inactive substance by adsorption onto an adsorbent resin.
5. A process as claimed in claim 3 or claim 4, wherein the producing microorganism belongs to the genus *Streptomyces*.
6. A process as claimed in claim 3, 4 or 5, wherein the producing 10 microorganism is *Streptomyces* NCIMB 40515.
7. A pharmaceutical composition comprising a compound according to claim 1 or 2, or mixture thereof, or a pharmaceutically acceptable derivative thereof, together with a pharmaceutically acceptable carrier or 15 excipient.
8. A compound according to claim 1 or claim 2, or mixture thereof, or a pharmaceutically acceptable derivative thereof, for use in therapy.
- 20 9. A compound according to claim 1 or claim 2, or mixture thereof, or a pharmaceutically acceptable derivative thereof, for use as an immunomodulatory agent as an anticancer agent or in the treatment of microbial infections in animals including humans.
- 25 10. Use of a compound according to claim 1 or claim 2, or mixture thereof, or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for use as an anticancer or immunomodulatory agent or in the treatment of microbial infections in animals including humans.
- 30 11. A method of immunomodulation of an animal or treating microbial infections in animals or treating carcinogenic tumours, especially in humans and in domesticated mammals, which method comprises administering a compound according to claim 1 or claim 2, or a mixture thereof, or a pharmaceutically acceptable derivative thereof or a 35 composition according to claim 7, to a patient in need thereof.
12. *Streptomyces* NCIMB 40515.

INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/GB 94/01600A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D498/18 A61K31/435 C12P17/18 C12R1/465

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D A61K C12P C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	US,A,5 310 901 (PARSONS) 10 May 1994 see claim 1 ---	1-10
P,A	US,A,5 310 903 (GOULET) 10 May 1994 see claim 1 ---	1-10
P,A	US,A,5 256 790 (NELSON) 26 October 1993 see claim 1 ---	1-10
P,A	EP,A,0 593 227 (AMERICAN HOME PRODUCTS CORP.) 20 April 1994 see claim 1 ---	1-10
A	GB,A,1 436 447 (AYERST,MCKENNA & HARRISON) 19 May 1976 cited in the application see claims 1-3 -----	1-10

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *B* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

& document member of the same patent family

1 Date of the actual completion of the international search

29 September 1994

Date of mailing of the international search report

13.10.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentiaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Gettins, M

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int'l Application No

PCT/GB 94/01600

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5310901	10-05-94	NONE		
US-A-5310903	10-05-94	NONE		
US-A-5256790	26-10-93	AU-B-	5007193	15-03-94
		WO-A-	9404540	03-03-94
		US-A-	5302600	12-04-94
EP-A-0593227	20-04-94	US-A-	5302584	12-04-94
		AU-B-	4894293	28-04-94
		BR-A-	9304211	07-06-94
		FI-A-	934501	14-04-94
		CA-A-	2108068	14-04-94
GB-A-1436447	19-05-76	AU-B-	474405	22-07-76
		AU-A-	6044773	20-03-75
		BE-A-	805505	28-03-74
		CA-A-	1000631	30-11-76
		CH-A-	591558	30-09-77
		DE-A,C	2347682	11-04-74
		FR-A,B	2201076	26-04-74
		NL-A-	7313386	02-04-74
		SE-B-	399437	13-02-78
		US-A-	3929992	30-12-75